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Published in:
Hearing Research

DOI:
10.1016/j.heares.2014.03.004

Hearing Research 312 (2014) 38–47

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

The dissimilar time course of temporary threshold shifts and reduction of inhibition in the inferior colliculus following intense sound exposure

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ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form
5 February 2014
Accepted 5 March 2014
Available online 18 March 2014

ABSTRACT

Excessive noise exposure is known to produce an auditory threshold shift, which can be permanent or transient in nature. Recent studies showed that noise-induced temporary threshold shifts are associated with loss of synaptic connections to the inner hair cells and with cochlear nerve degeneration, which is reflected in a decreased amplitude of wave I of the auditory brainstem response (ABR). This suggests that, despite normal auditory thresholds, central auditory processing may be abnormal.

We recorded changes in central auditory processing following a sound-induced temporary threshold shift. Anesthetized guinea pigs were exposed for 1 h to a pure tone of 11 kHz (124 dB sound pressure level). Hearing thresholds, amplitudes of ABR waves I and IV, and spontaneous and tone-evoked firing rates in the inferior colliculus (IC) were assessed immediately, one week, two weeks, and four weeks post exposure.

Hearing thresholds were elevated immediately following overexposure, but recovered within one week. The amplitude of the ABR wave I was decreased in all sound-exposed animals for all test periods. In contrast, the ABR wave IV amplitude was only decreased immediately after overexposure and recovered within a week. The proportion of IC units that show inhibitory responses to pure tones decreased substantially up to two weeks after overexposure, especially when stimulated with high frequencies. The proportion of excitatory responses to low frequencies was increased. Spontaneous activity was unaffected by the overexposure.

Despite rapid normalization of auditory thresholds, our results suggest an increased central gain following sound exposure and an abnormal balance between excitatory and inhibitory responses in the midbrain up to two weeks after overexposure. These findings may be associated with hyperacusis after a sound-induced temporary threshold shift.

1. Introduction

Exposure to loud noises may result in tinnitus, i.e. an acoustic perception in the absence of an external sound source, and/or in hyperacusis, a phenomenon defined as over-sensitivity to acoustic input. However, the most common consequence of excessive exposure to noise is an elevation of hearing thresholds. The elevation of thresholds may be permanent or temporary. Noise exposure associated with a permanent threshold shift results in a variety of pathologies in the peripheral and central auditory pathway.

Peripherally, both inner and outer hair cells may degenerate after noise exposure (Salvi et al., 2000). Furthermore, a number of structural changes have been observed. Synaptic ribbons in the

Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; CF, characteristic frequency; fMRI, functional magnetic resonance imaging; GABA, γ-aminobutyric acid; IC, inferior colliculus; PSTH, post-stimulus time histogram; RM-ANOVA, repeated measures analysis of variance; SEM, standard error of the mean; SPL, sound pressure level; SpO_2, blood oxygen saturation

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http://dx.doi.org/10.1016/j.heares.2014.03.004

surviving inner hair cells, which are important for spike reliability in the auditory nerve, are reduced in number (Zuccotti et al., 2012). Afferent dendrites that innervate the hair cells may swell, and spiral ganglion cells degenerate (Duan et al., 2000). These phenomena explain the permanently elevated hearing thresholds after noise exposure. Subsequently, along the central auditory pathway, γ-aminobutyric acid (GABA) inhibitory neurotransmission in the inferior colliculus (IC) is reduced (Dong et al., 2010a; Milbrandt et al., 2000), whereas the strength of excitatory responses in the IC is increased (Willott and Lu, 1982; Niu et al., 2013). In the auditory cortex, the balance between excitation and inhibition is likewise disrupted (Scholl and Wehr, 2008). Moreover, spontaneous firing rates of units in the IC are altered following sound-induced hearing loss (Mulders and Robertson, 2009; Niu et al., 2013). It is thought that these central pathologies, among others, are correlated with tinnitus and hyperacusis (Eggermont and Roberts, 2004; Knipper et al., 2013).

These changes occur after a noise-induced permanent elevation of the hearing thresholds. However, tinnitus and hyperacusis can also occur in the absence of permanent elevated hearing thresholds, suggesting abnormalities in the central auditory system. Indeed, in tinnitus patients with normal hearing thresholds, it has been shown that the amplitude of wave I of the auditory brainstem response (ABR) is reduced, whereas the amplitude of ABR wave V remains unchanged (Schaette and McAlpine, 2011). This indicates an increased neural gain, since a reduced auditory nerve response (wave I) must be enhanced in the brainstem to produce a normal auditory midbrain response (wave V). Further evidence of increased central gain came from a functional magnetic resonance imaging (fMRI) study, that showed sound-evoked hyperactivity of the IC in hyperacusis patients with normal audiograms (Gu et al., 2010).

Recently, it has been shown that the peripheral auditory system may also be permanently damaged by a noise exposure that is only associated with a temporary threshold shift. Following recovery of hearing thresholds, the amplitude of ABR wave I is chronically reduced, despite apparently unaffected hair cell integrity. This reduction of wave I is associated with a 50% loss of afferent nerve terminals on inner hair cells, and with disorganization and a reduced number of synaptic ribbons. Furthermore, within months, the auditory nerve slowly degenerates. Specifically, auditory nerve fibers that have high thresholds and low spontaneous firing rates are affected more than those with low thresholds and high spontaneous rates (Furman et al., 2013; Kujawa and Liberman, 2009; Lin et al., 2011).

At present it is not known to what extent conditions that may affect the peripheral hair cell synapse influence response properties in the central auditory system. It is conceivable that damage to the peripheral synapse, such as a loss of synaptic ribbons, results in changes in central auditory processing, despite the presence of normal hearing thresholds. The current study aims at determining possible abnormalities in central auditory processing caused by sound exposures that are associated with rapidly recovering hearing thresholds. We assessed ABR waveforms after acoustic trauma that induced temporary threshold elevations in guinea pigs. These results are readily comparable to those in human subjects (Schaette and McAlpine, 2011). In addition, multi-unit recordings were made in the IC and provide a more detailed insight into excitatory and inhibitory responses in the auditory midbrain. Finally, the recordings of spontaneous neural activity provide a possible correlate of tinnitus.

2. Materials and methods

2.1. Experimental groups

Fifteen male albino guinea pigs (Dunkin Hartley; Harlan Laboratories, Horst, the Netherlands) were used in this study. Guinea pigs weighed between 250 and 300 g upon arrival in the Central Animal Facility of the University Medical Center Groningen, where they were socially housed. Sound pressure levels (SPLs) in the housing room did not exceed 65 dB SPL (36 dB A). All neurophysiology was carried out six weeks after arrival, to ensure that all guinea pigs had approximately the same age and weight during recording of IC activity. Guinea pigs were exposed to a loud tone either four weeks ($n = 3$), two weeks ($n = 3$), one week ($n = 3$), or immediately ($n = 3$) before neurophysiology. In addition, a control/sham group ($n = 3$) was treated similarly, except for the sound exposure, two weeks before the neurophysiology. Thus all guinea pigs were allowed to acclimatize to laboratory conditions for at least two weeks before experimental procedures started (Fig. 1A). All experiments were approved by the Animal Experiment Committee of the University of Groningen (DEC # 6068B) and were in compliance with Dutch and European law and regulations.

2.2. Sound exposure

Guinea pigs that were tone- or sham-exposed four weeks, two weeks, or one week before neurophysiology were anesthetized with isoflurane (5% for initiating and 2.5% for maintenance of anesthesia) in a mixture of medical air and oxygen. Animals that were exposed to the tone immediately before neurophysiology were anesthetized with ketamine/xylazine (70 mg/kg Ketamine, Alfasan, Woerden-Holland; 6 mg/kg Rompun (xylazine), Bayer-Healthcare, respectively). Heart rate and blood oxygen saturation (SpO_2) were monitored using a pulsoximeter. Body temperature was held constant at 38 °C by a heating pad. Two Piezo tweeters (PH8; Velleman) were positioned at approximately 5 cm from each ear, resulting in free field overexposure. Both pinnae were folded over the head, to create an unobstructed path from the speakers to the tympanic membrane. Animals were bilaterally exposed to a continuous tone of 11 kHz (124 dB SPL) for 1 h. The trauma stimulus was designed in RPvdsEx (Tucker–Davis Technologies; TDT Inc.), generated by a Real-Time Processor (RP2.1, TDT Inc.) and amplified (Philips PM 5170 amplifier). A measuring microphone (Bruël & Kjær; type 2670) and amplifier (Bruël & Kjær; type 2610) were used to calibrate the stimulus level at the entrance of the ear canal. The sham-exposure group was treated as the other groups, without the amplifier being connected. All subsequent stimulus levels will be expressed as dB SPL.

2.3. Neurophysiology

2.3.1. Auditory brainstem responses (ABRs)

ABRs were collected before and immediately after sound exposure, and on the day of the recordings from the IC. Anesthetics and monitoring of the animal were as described in Section 2.2. The stimuli were 3 ms pure tones (3, 6, 11, and 22 kHz; 0.2 ms $\cos^2$ on and off ramps) and a 0.1 ms click that were designed in SigGenRP. Stimulus level started at 100 dB and decreased in steps of 10 dB. All stimuli were presented 1000 times at each level with a repetition rate of 33/s. Presentation was controlled by BioSigRP software (TDT Inc.). Acoustic stimuli were generated by a Real-Time processor (RP2.1, TDT Inc.) and an attenuator (PA5, TDT Inc.), and presented via a free field electrostatic speaker driver and speaker (ED1 and ES1, TDT Inc.). The speaker was placed at approximately 2 cm in front of the nose of the animal. Stimuli were calibrated using a B&K microphone (type 2670) and amplifier (type 2610). Electrodes were placed subdermally at the vertex, behind the ipsilateral pinna, and behind the contralateral pinna, for reference, recording, and grounding, respectively. ABR signals were amplified (25K times) and filtered (0.3–3 kHz, −6 dB/octave slope) by a pre-amplifier (EG

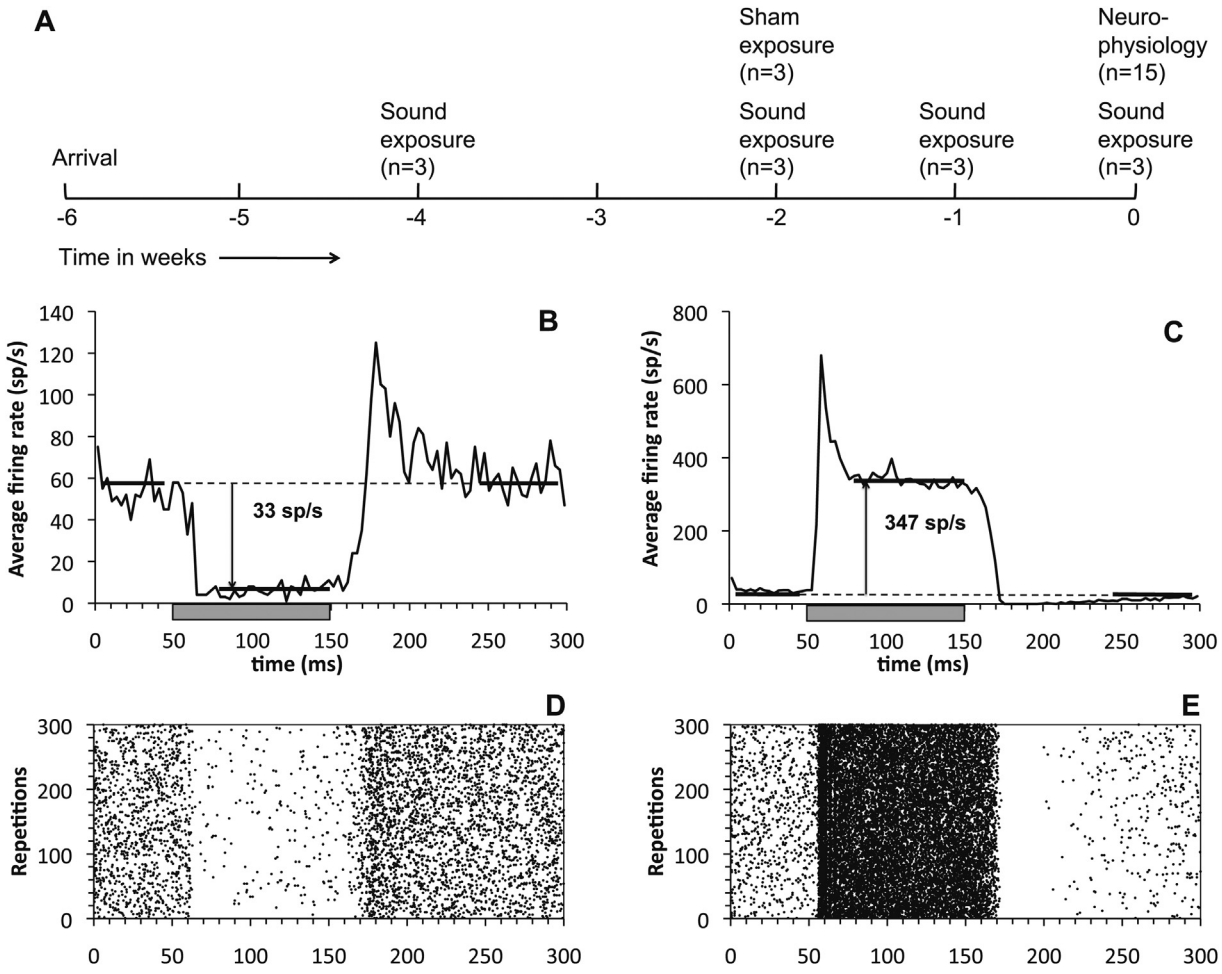

Fig. 1. A) Experimental design. Neurophysiology was always performed six weeks after arrival in the animal facility. Time of sound exposure differed between the groups: either four weeks, two weeks, one week, or immediately before neurophysiology. Sham exposure of the control group took place two weeks before neurophysiology. ABRs were recorded directly before and after sound exposure, and on the day of neurophysiology. B) – E) Examples of sound-evoked activity. Averaged stimulus-driven firing rate in sp/s (spikes/sec) over 300 presentations of a 80 dB 22-kHz pure tone (gray bar) in a sham-exposed animal. PSTH (B) and its raw data traces (D) of a significant inhibitory response of an IC multi-unit (CF 8.9 kHz; threshold 37 dB SPL) with a response strength of 33 sp/s. PSTH (C) and its raw data traces (E) of a significant excitatory response of an IC multi-unit (CF 21.5 kHz; threshold 39 dB SPL) with a response strength of 347 sp/s. Horizontal black bars in panel B and C show how the response strength is calculated.

&G Inc.; model 5113), recorded by a second RP2.1, and saved on a PC using BioSigRP software.

2.3.2. Extracellular multi-unit recordings from the inferior colliculus

In vivo neurophysiological recordings from the IC were performed in a sound-attenuating booth either four weeks, two weeks, one week, or immediately after sound exposure (Fig. 1A). Neurophysiology of the control group was recorded two weeks after sham exposure. Animals were anesthetized with a mixture of ketamine and xylazine (70 mg/kg and 6 mg/kg, respectively; i.m.). To maintain a deep level of anesthesia, supplementary injections with half the original dose were administered every hour. A tracheotomy was performed for artificial respiration and a skull screw used for fixation of the head. Next, a craniotomy was made above the right IC. The head of the animal was slightly turned around the rostro–caudal axis, to position the craniotomy horizontally. The dura mater below the craniotomy was removed and cortical brain tissue aspirated, to allow visual placement of a single-shank 16-channel microelectrode array in the right IC. The electrodes in the array were arranged in a single column along the axis of penetration, were 100 μm apart, and had a contact surface equal to 413 μm^2 (A1x16-10 mm-100-413-A16; NeuroNexus). The microelectrode array was inserted into the IC in the lateral-dorsal to medial-ventral direction, in order to obtain recordings from a broad tonotopic gradient.

Acoustic stimuli were generated (RP2.1; TDT Inc.), attenuated (PA5; TDT Inc.), and presented (ED1, ES1; TDT Inc.) at ±5 cm from the contralateral ear. Stimuli were calibrated using a B&K microphone (type 2670) and amplifier (type 2610) placed at the entrance of the ear canal. Multi-unit neural activity was recorded using TDT hardware (preamplifier RA16PA and processor RX5) and software (RPvdsEx). MatLab programs (R2010b, MathWorks) were custom-made to store neural recordings and generate acoustic stimuli. Noise bursts (delay 50 ms, duration 100 ms, 10 ms $\cos^2$ gate) were presented while the microelectrode array was slowly inserted into the IC, using a micromanipulator (Kopf instruments). Real-time inspection of multi-unit activity before, during, and after the noise bursts revealed which channels on the microelectrode recorded from auditory neurons, and this was used for optimal placement in the IC.

Pure tones with a frequency of 3, 6, 11, and 22 kHz (delay 50 ms, 10 ms $\cos^2$ ramp, duration 100 ms, 300 ms recording time, 80 dB, 300 repetitions) were presented to elicit acoustically evoked activity, which was used to plot post-stimulus time histograms (PSTHs). In addition, receptive fields were obtained by recording neural activity resulting from stimulation with 300 ms pure tones

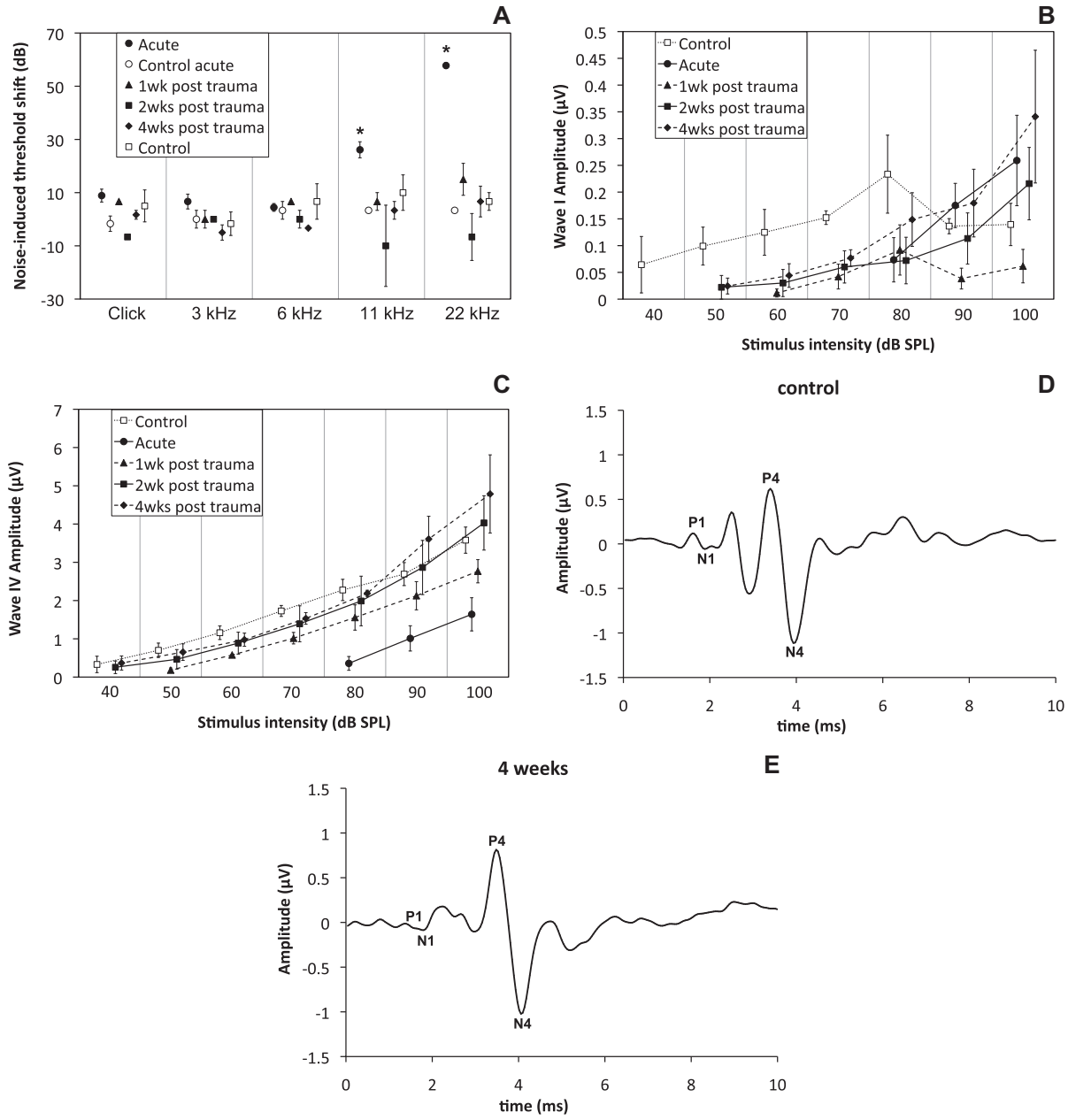

Fig. 2. ABR recordings. A) ABR threshold shifts. Tone-induced ABR threshold shifts ± SEM measured immediately after sound exposure (closed circles) are significantly increased compared to ABR threshold shifts following sham exposure (open circles) at 11 kHz and at 22 kHz (see *; RM-ANOVA, $F = 10.629$, $p < 0.001$, one-sample t-test with Bonferroni correction for multiple testing). Sound-induced threshold shifts one week (closed triangles), two weeks (closed squares), and four weeks (closed diamonds) after exposure did not differ from two weeks after sham exposure (open squares). B) Wave I amplitudes. Average amplitudes ± SEM (in μV) of the ABR wave I of the tone-exposed groups (closed markers) up to 80 dB stimulus level are significantly different from the control group (open squares; RM-ANOVA, $F = 5.910$, $p < 0.01$). Groups did not differ at levels >80 dB (RM-ANOVA, ns). C) Wave IV amplitudes. The average amplitude ± SEM (in μV) of ABR wave IV of the group measured immediately following sound exposure (closed circles) is significantly different from the control group (open squares; RM-ANOVA, $F = 5.338$, $p < 0.05$). Examples of an ABR trace in response to a 22-kHz tone of 70 dB from a sham-exposed guinea pig (D) and a guinea pig measured four weeks post exposure (E). These examples demonstrate the permanently reduced wave I amplitude (P1–N1) and recovered wave IV amplitude (P4–N4) four weeks after sound exposure.

of different frequencies (2–40 kHz, 25 steps, logarithmically spaced) and levels (0–80 dB, 15 steps, linearly spaced), presented randomly. Plotting the receptive field enabled us to determine the characteristic frequency (CF), i.e. the frequency at which the unit responds to the lowest sound level, and the corresponding threshold (Duque et al., 2012). And finally, two recordings without acoustical stimulation were acquired (180 s duration), to assess spontaneous activity.

2.4. Data analysis

ABR thresholds were determined by visual inspection and were considered the lowest stimulus level for which ABR wave IV, the most prominent and consistent waveform in our measurements, was clearly detectable. The tone-induced threshold shift was defined as the difference between the ABR threshold before and after the sound (or sham) exposure, and was calculated for every

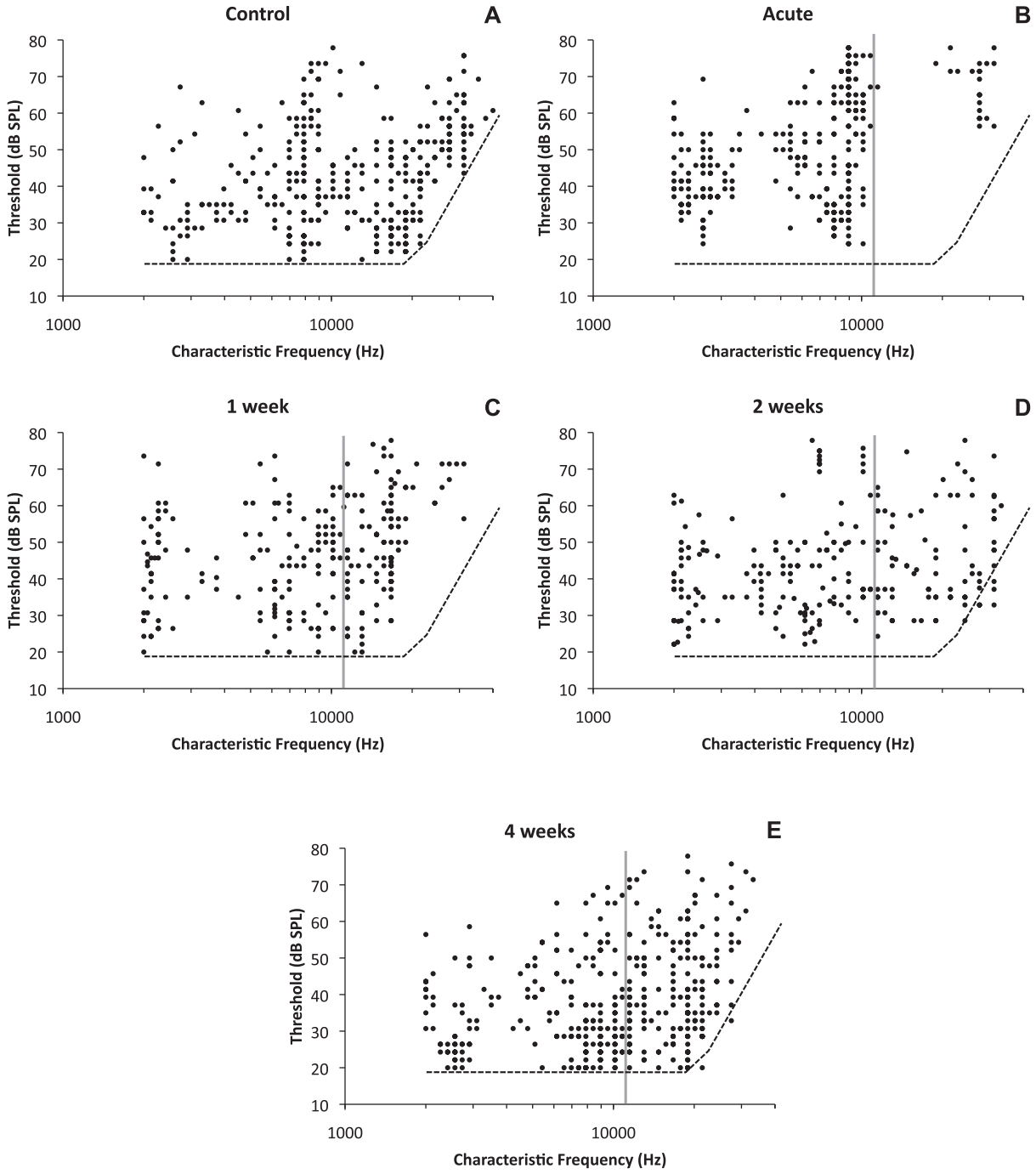

Fig. 3. IC multi-unit CFs and thresholds. The CF and threshold of all IC multi-units that were included in the study for the control group (A), and the group measured immediately (B), one week (C), two weeks (D), and four weeks (E) following sound exposure. The vertical gray line depicts the frequency of the exposure (11 kHz). The dashed line indicates the lowest thresholds measured in the sham-exposed group.

animal individually. Furthermore, the amplitudes of wave I and wave IV were determined by calculating the difference between P1 and N1, and between P4 and N4, respectively (Fig. 2D shows an example of an ABR to 22 kHz, 70 dB tones in a sham-exposed guinea pig). The waveform amplitudes (in μV) were determined in the ABRs that were recorded on the day of neurophysiology. Only wave amplitudes in response to 22 kHz were taken into account, because waveform abnormalities are expected in ABRs in response to stimulation with tones one octave above the exposure frequency (Kujawa and Liberman, 2009).

Neural data were analyzed using custom-made MatLab programs (R2010b, MathWorks). To set a threshold for spike detection, the root-mean-square of filtered signals (300–3000 Hz, butterworth filter) was calculated and multiplied by three. Artifacts were removed using a custom-made algorithm. Units that had a clear response to white noise and a distinguishable receptive field were included in further analyses. Also, units without a clear receptive field, but that were recorded at an electrode located within 600 μm ventrally from a unit with a CF $<$ 8 kHz, were included. This last criterion included auditory IC

neurons lacking a clear receptive field due to damage by sound exposure.

The proportion of units that suppressed or enhanced activity upon stimulation with pure tones and the respective mean change in spike rate were taken as a measure of inhibition and excitation, respectively. These measures were obtained from the PSTH, which was acquired between 0 and 300 ms, where the stimuli were presented between 50 and 150 ms. First, firing rates in the time windows 5–45 ms and 250–295 ms over all four recordings of that unit were averaged. Subsequently, the stimulus-evoked activity was calculated per stimulus by averaging activity over all 300 repetitions to that stimulus in the time window 80–150 ms and compared with the previously calculated firing rate before and after the stimulus. Thus, on-responses, i.e. the first response of a unit to an acoustic stimulus, were excluded in these analyses by starting the time window at 30 ms after onset of the stimulus. Significance of response strengths was determined by a Wilcoxon rank sum test and $p < 0.001$ was considered significant. When the activity during stimulus presentation was not significantly different from firing rates before and after the stimulus, it was referred to as a 'no response'. Fig. 1 shows an example of PSTHs of an inhibitory (panel B) and an excitatory (panel C) response to a pure tone of 22 kHz in a sham-exposed animal, and the corresponding time windows that were used to determine response strengths. Panel D and E of Fig. 1 show the raw traces of the same data of the inhibitory and excitatory responses, respectively. The unit's CF and its corresponding threshold were determined by visual inspection of the receptive fields. Spontaneous firing rates were calculated by averaging firing rates of the two data files recorded in the absence of acoustic stimulation.

2.5. Statistics

Significance of ABR threshold shifts was calculated by a repeated-measures analysis of variance (RM-ANOVA; stimulus level as within-factor and group as between-factor), using a Bonferroni correction for multiple comparisons (IBM SPSS Statistics; Version 19). Differences in proportions of responses between the control group and the experimental groups were tested using a two-tailed bootstrapping test. This method made a probability distribution of the proportion of, for example, inhibitory responses under the assumption that the proportion of such a response is equal to that in the control animals (null hypothesis). Then, it calculated the chance that the experimental value was derived from that distribution ($p < 0.05$ was considered significant after a Bonferroni correction). Differences between groups in firing rate changes and spontaneous activity were determined using a one-way analysis of variance (ANOVA), with a Bonferroni correction. Experimental groups were only compared to the control group, and not to each other, in all statistic tests.

3. Results

3.1. Hearing thresholds

Tonal exposure resulted in an immediate threshold shift of 30 dB at the exposure frequency (11 kHz) and of 60 dB one octave above the exposure frequency (Fig. 2A; RM-ANOVA: immediately following exposure "Acute" vs. immediately following sham exposure "Control acute", $F = 10.629$, $p < 0.001$, one-sample t-test with a Bonferroni correction). Thresholds recovered completely within one week following overexposure (RM-ANOVA, ns).

In addition to the ABR thresholds, we also studied the thresholds and the CFs of IC units. In sham-exposed animals, 52.5% of the recorded IC units had a CF above 11 kHz (Fig. 3A) with thresholds ranging from 20 to 75 dB SPL. Immediately following overexposure, the percentage of recorded units with a CF above 11 kHz decreased to 6.0%, all with thresholds higher than 55 dB SPL. One week following overexposure, the proportion of units tuned to 11 kHz or higher had recovered to 43.2% (Fig. 3C). Thresholds of units with CF $\geq$ 22 kHz were still elevated $\pm$20 dB compared to control levels. From two weeks after overexposure, CFs and thresholds were similar to control levels (Fig. 3D and E). Some IC units, however, did not show a distinguishable receptive field. The numbers of these units were 2, 87, 21, 25, and 8 for the control, acute, one-week, two-weeks, and four-weeks group, respectively.

3.2. ABR wave amplitudes

The amplitude of wave I of the ABR was decreased in every experimental group at stimulus levels $\leq$80 dB compared to the control group (Fig. 2B; RM-ANOVA, $F = 5.910$, $p < 0.05$). However, for stimulus levels higher than 80 dB, the amplitude of wave I of control animals did not increase further, it rather stabilized at a lower amplitude. Contrarily, wave I amplitudes of the experimental groups monotonically increased with increasing stimulus level. There were no significant differences between the groups when only stimulus levels >80 dB were taken into account (RM-ANOVA, ns).

Wave IV amplitudes were decreased for all stimulus levels when measured immediately after overexposure, but recovered within one week (Fig. 2C; RM-ANOVA, $F = 5.338$, $p < 0.05$). See Fig. 2D and E for representative examples of an ABR of the control group and of an ABR of the four-weeks post exposure group, respectively. Note the decreased wave I amplitude (P1 − N1) and recovered wave IV amplitude (P4 − N4) in the experimental group in panel E compared to panel D (sham-exposed animal).

3.3. Proportions of stimulus-evoked IC responses

Fig. 4 displays how all recorded multi-unit responses in the IC were divided between no responses, inhibitory responses, and excitatory responses for stimulation with pure tones of 3 kHz, 6 kHz, 11 kHz, and 22 kHz (panel A–D, respectively) for all experimental groups. The proportion of inhibitory responses to all frequencies decreased significantly immediately following overexposure (two-tailed bootstrap test; control vs. acute group for all tested frequencies, $p < 0.05$). Furthermore, the proportion of excitatory responses significantly increased for stimulation with 3 kHz and 6 kHz, but decreased for 22 kHz. One week after overexposure, the proportion of inhibitory responses was still significantly reduced when stimulated with 3 kHz, 6 kHz, and 22 kHz. Also, the proportion of excitatory responses was increased when stimulated at the exposure frequency and one octave below the exposure frequency. Two weeks post trauma, the proportion of inhibitory responses to 6 kHz and 22 kHz was still slightly, but significantly, decreased, whereas the proportion of excitatory responses to 6 kHz was increased. Four weeks after overexposure, proportions of responses to 22 kHz were recovered. In addition, when stimulated with 3-kHz and 6-kHz pure tones, the proportion of excitatory responses was increased and the proportion of inhibitory responses was decreased. Pure tones of 11 kHz elicited more excitatory responses compared to the control group.

3.4. Amplitude of stimulus-evoked IC responses

Immediately after overexposure, amplitudes of inhibitory responses to 11-kHz and 22-kHz tones, as measured by suppression

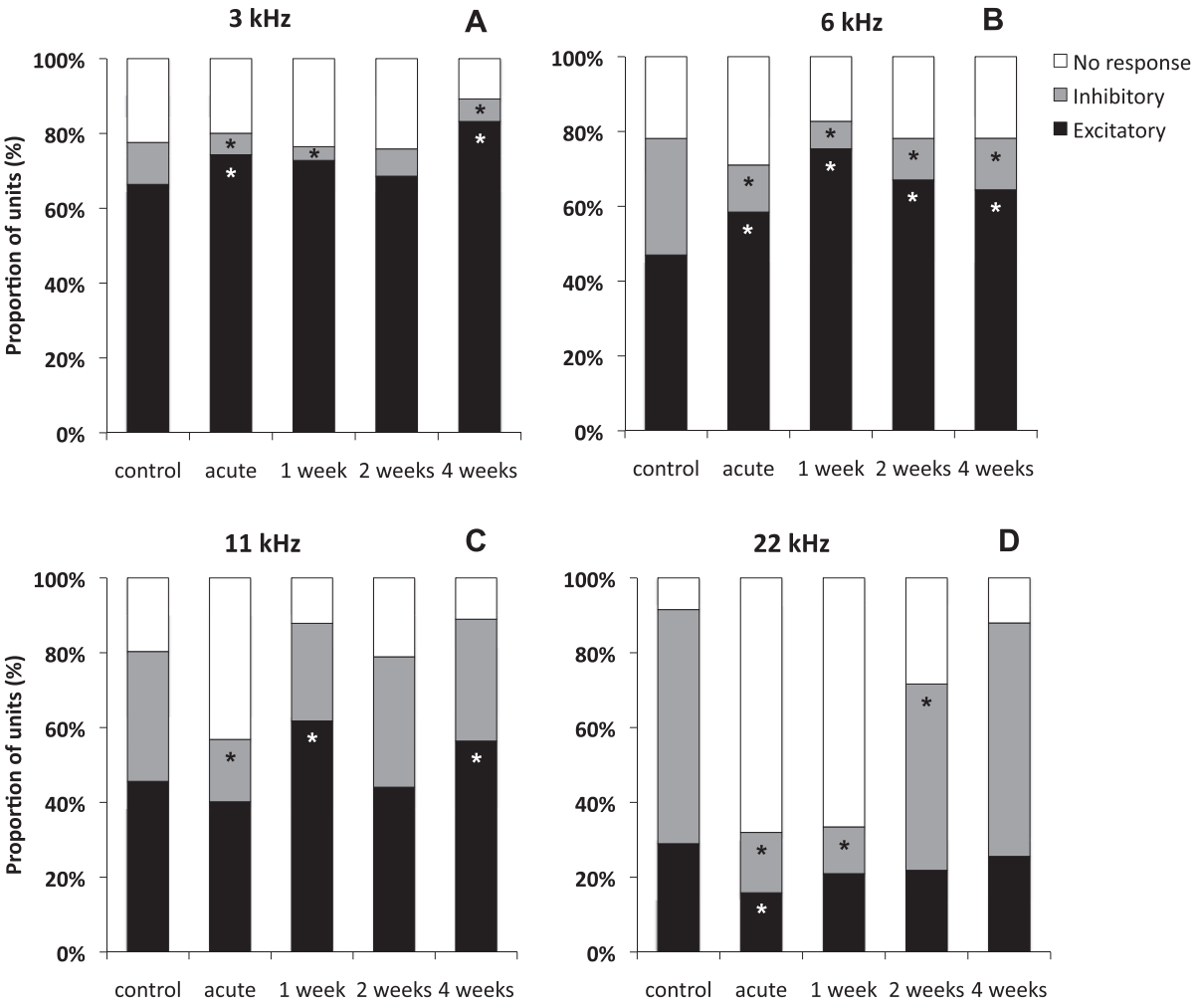

Fig. 4. Proportions of stimulus-driven response types. The distribution of units between 'no responses' (white bars), 'inhibitory responses' (gray bars), and 'excitatory responses' (black bars) is shown for the five groups when stimulated with pure tones of 3 kHz (A), 6 kHz (B), 11 kHz (C), and 22 kHz (D). An '*' indicates a significant difference compared to the control condition, as determined by a two-tailed bootstrap test, corrected for multiple testing. See Fig. 1 for examples of a PSTH and its raw data traces of an inhibitory response (Fig. 1B and D, respectively) and of an excitatory response (Fig. 1C and E, respectively).

of firing rate, were decreased as compared to the control group (Fig. 5C and D, respectively; One-way ANOVA $F = 18.752$, $p < 0.001$; One-way ANOVA $F = 30.860$, $p < 0.001$, respectively). Similarly, the amplitudes of excitatory responses to 11 kHz and 22 kHz tones were also decreased immediately after overexposure (One-way ANOVA $F = 14.818$, $p < 0.001$; One-way ANOVA $F = 23.717$, $p < 0.001$, respectively). One week after overexposure, amplitudes of inhibitory responses to both 11 kHz and 22 kHz remained decreased. Moreover, amplitudes of excitatory responses to 22 kHz also remained decreased. Two weeks after overexposure, amplitudes of inhibitory responses to 22 kHz were still significantly decreased. Furthermore, amplitudes of excitatory responses to 3 kHz tones were significantly increased compared to the control group (Fig. 5A; One-way ANOVA, $F = 14.237$, $p < 0.001$). Four weeks following overexposure, all responses were recovered, except that amplitudes of inhibitory responses to 3-kHz tones were significantly increased compared to the control group (One-way ANOVA, $F = 4.976$, $p < 0.001$). Throughout all time points measured, the amplitude of both inhibitory and excitatory responses to a 6-kHz tone did not significantly differ from the control group (Fig. 5B; One-way ANOVA, ns).

3.5. Spontaneous activity

There were no effects of sound exposure on spontaneous firing rates of IC units at any measured time point (Fig. 6A; One-way ANOVA, ns). Moreover, when only units with a CF above 11 kHz were taken into account, there were also no differences between any tone-exposed group and the control group (Fig. 6B; One-way ANOVA, ns).

4. Discussion

This study showed that a sound-induced temporary threshold shift was associated with a chronic reduction of ABR wave I amplitude, a temporary reduction of ABR wave IV amplitude, and a temporary reduction of tone-evoked inhibition in the IC. Although ABR thresholds recovered within one week, central auditory parameters required two to four weeks for full recovery or did not recover at all within four weeks.

Typically, the exposure used in this study has been described to cause a permanent elevation of hearing thresholds (e.g. Mulders and Robertson, 2009). Yet, in our study, thresholds were only

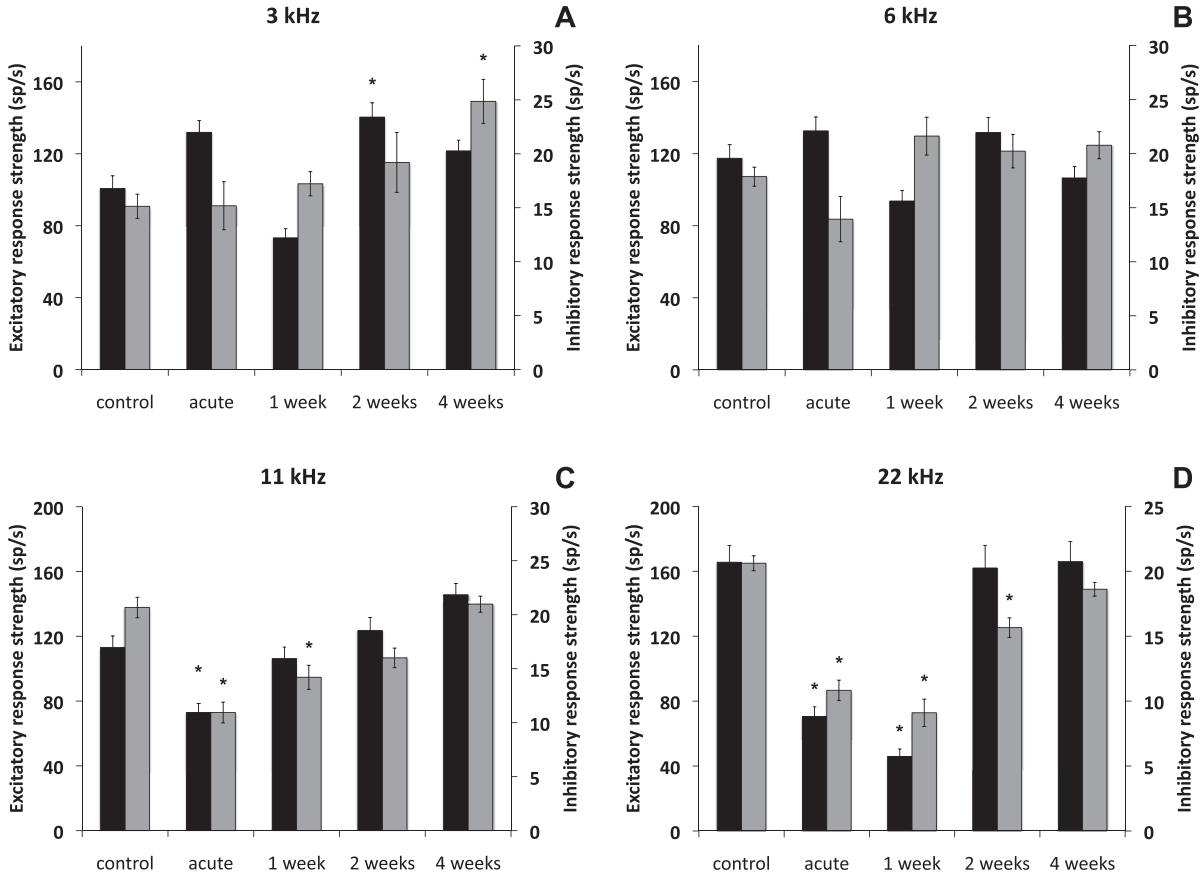

Fig. 5. Amplitudes of stimulus-driven activity. Amplitudes (in sp/s ± SEM) of responses to pure tones of 3 kHz (A), 6 kHz (B), 11 kHz (C), and 22 kHz (D). The left vertical axis depicts mean amplitude of excitatory response in sp/s (black bars), the right vertical axis depicts mean amplitude of inhibitory response in sp/s (gray bars). Note that the amplitude of excitatory responses is an enhancement in firing rate, whereas the amplitude of inhibitory responses is a suppression of firing rate. An '*' indicates a significant difference between that experimental group and the control group, as determined by a One-way ANOVA ($p < 0.001$).

briefly elevated. The rapid recovery of the thresholds, however, might be associated with the fact that the exposure was free field and bilateral. In contrast, the studies that report a permanent threshold shift up to twelve weeks after a similar exposure applied unilateral exposure (Mulders and Robertson, 2011). Hypothetically, the free-field (bilateral) trauma stimulus used in the current study activated the efferent auditory system (Buño, 1978; Liberman, 1989), which may protect against sound exposure (Maison and Liberman, 2000; Zheng et al., 1997).

Despite the rapidly recovered hearing thresholds, the central responses showed clear abnormalities, some of which were temporary and others permanent. The ABRs suggest that different mechanisms were in place at high level (>80 dB) vs. low to moderate level (≤80 dB) stimuli. Remarkably, the control group showed a reduction in wave I amplitude to high levels (>80 dB) when compared to levels ≤80 dB, which was not present in the traumatized animals. Purely speculatively, this could indicate a nonlinear protective mechanism, possibly mediated by the efferent system (Maison and Liberman, 2000; Zheng et al., 1997). That mechanism could suppress the output of the cochlea to the auditory nerve, resulting in a reduced ABR wave I amplitude at high-level stimuli. The absence of this effect in traumatized guinea pigs suggests that the proposed protective mechanism may be damaged by sound exposure. Furthermore, an effect of time can be observed at high levels, in which the time after sound exposure is positively correlated with wave amplitude, suggesting that the damage progresses in the weeks following the trauma (see Fig. 2B).

For the lower levels (≤80 dB), the amplitude of wave I is reduced at all measured time points following sound exposure, in spite of recovered hearing thresholds. On the other hand, wave IV amplitude had recovered when the thresholds were recovered. This indicates that a central mechanism increased the neural gain between the cochlear nerve (wave I) and the auditory midbrain (wave IV), similar to what happens in tinnitus patients with normal thresholds (Schaette and McAlpine, 2011).

The multi-channel recordings in the IC showed a decrease in inhibition, both in strength and in proportion, immediately following overexposure. This was observed in response to all tested frequencies, even in response to tones outside the range of the temporary threshold shift. Inhibition gradually recovered over the four-week period following sound exposure. Our findings confirm studies that show disrupted inhibition in the IC at a molecular level following noise exposure (Dong et al., 2010a, 2010b; Szczepaniak and Møller, 1995).

As mentioned previously, the chronic reduction of wave I amplitude suggests degeneration of high-threshold cochlear-nerve fibers (Furman et al., 2013). ABR thresholds are obviously mediated by low-threshold fibers; hence the recovered thresholds suggest that any damage to the low-threshold fibers recovered after a week. Therefore, disrupted inhibition in the IC might have been a result of damage to high-threshold fibers. It can be speculated that high-threshold fibers (indirectly) innervate inhibitory projections in the IC, as these are triggered by high level stimuli (Ehret and Romand, 1997). This would explain why inhibition was abnormal

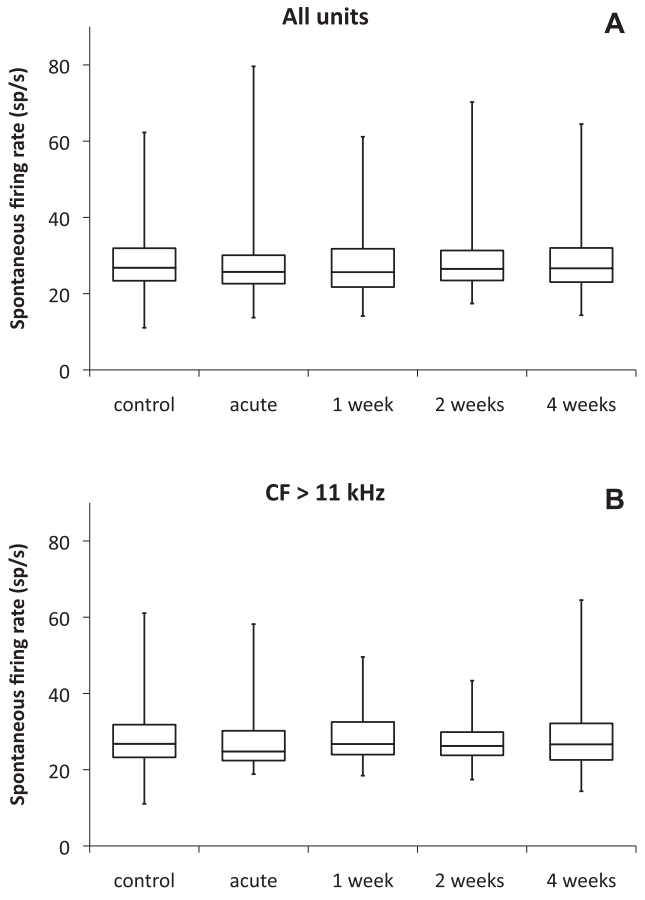

Fig. 6. Box-plots of spontaneous multi-unit activity. The horizontal line in the box represents the median, the upper and lower borders of the box represent the 25%- and 75%-quartiles, and the error bars represent the maximum and minimum values of the data. A) Average spontaneous multi-unit activity (in sp/s) of all units (One-way ANOVA; ns). B) Average spontaneous multi-unit activity of units with a CF higher than 11 kHz (One-way ANOVA; ns).

when thresholds were already recovered, and demonstrates the presence of a 'hidden' hearing loss. This hearing loss is hidden in the sense that it is not revealed by a hearing threshold measure.

Conversely, tone-evoked excitatory responses increased in number and strength as a result of the sound exposure, especially in response to low-frequency tones. This finding is confirmed by other electrophysiological studies (Salvi et al., 1990; Sun et al., 2012) and in an fMRI study of tinnitus patients (Langers et al., 2012). Taken together, our results indicate an imbalance between inhibition and excitation in the IC, seen more prominently in the proportions than in response strengths.

We showed that free-field exposure did not change spontaneous firing rates in the IC. Previous studies report mixed effects of noise trauma on spontaneous activity. It has been reported to decrease (Salvi et al., 1978; Niu et al., 2013), to be unchanged (Wang et al., 1996), or to increase (Dong et al., 2010b; Ma et al., 2006) following sound exposure. It has been shown that, specifically, units tuned to frequencies at and above the trauma frequency can develop increased spontaneous firing rates (Mulders and Robertson, 2009). Selectively comparing high-frequency units of exposed animals with the control group, however, also did not reveal any significant differences. The absence of increased spontaneous firing rates, even though inhibition was reduced, is further supported by Dong and colleagues, who demonstrated a decreased expression of the α-subunit of GABA receptors two weeks after a temporary noise-induced threshold shift (Dong et al., 2010a). This subunit is specific for GABA-A receptors, which are ionotropic receptors mainly involved in stimulus-driven inhibition, but not in spontaneous activity.

In summary, our main findings point to an increased gain in the central auditory system, which followed a different time course compared to the tone-induced threshold shift. The increased central gain was reflected in recovered ABR wave IV amplitudes, while wave I amplitudes were chronically reduced. Furthermore, a substantial proportion of inhibition in the IC was temporary reduced, whereas excitation to low frequencies was slightly increased. Even though increased central gain was evident in stimulus-evoked activity, spontaneous activity was not affected.

We propose that temporary threshold shifts, induced by sound exposure, are associated with changes in the central auditory system that are involved in hyperacusis. A recent review by Knipper et al. (2013) hypothesizes that "an over-adaptive compensating central gain that spreads from the brainstem toward ascending pathways may be associated with hyperacusis, but not with tinnitus" (Knipper et al., 2013). In addition, modeling of compensatory mechanisms of the central auditory system revealed that central gain corresponds to hyperacusis, whereas central noise, i.e. spontaneous activity, corresponds to tinnitus (Zeng, 2013). This model is supported by previous studies using animal models, which show that mechanisms of tinnitus are manifest in altered and/or elevated spontaneous activity (Eggermont and Roberts, 2004), whereas hyperacusis has been suggested to be correlated with a disruption of stimulus-driven activity (Eggermont, 2013). Furthermore, noise-induced synaptic ribbon loss in mice, in the absence of a permanent threshold shift, is associated with behavioral measures of hyperacusis, but not with behavioral measures of tinnitus (Hickox and Liberman, 2014). In humans, hyperactivity of the IC has also been linked to hyperacusis, in the absence of a threshold shift (Gu et al., 2010). The increased amplitudes of wave I and wave IV at high levels after sound exposure point to an increased stimulus-evoked activity in the brainstem, which is also indicative of hyperacusis.

5. Conclusion

This study reports on the consequences of acoustic overexposure on central auditory processing. We showed that a temporary threshold shift, due to free field overexposure, was associated with long-term central plasticity, expressed in disrupted stimulus-driven inhibition and enhanced excitation to low frequencies. We propose that these findings point to an underlying mechanism for hyperacusis.

Acknowledgments

The authors want to thank Hans Segenhout for technical support and Russ Snyder for showing us the surgical procedures involved in the IC recordings. We thank Geoffrey Manley for providing valuable suggestions for the English language in the manuscript.

This work was supported by the Heinsius Houbolt Foundation and the Stichting Gehoorgestoorde Kind. The study is part of the research program of our department: Healthy Aging and Communication.

References

Buño Jr., W., 1978. Auditory nerve fiber activity influenced by contralateral ear sound stimulation. Exp. Neurol. 59 (1), 62–74.

Dong, S., Rodger, J., Mulders, W.H.A.M., Robertson, D., 2010a. Tonotopic changes in GABA receptor expression in guinea pig inferior colliculus after partial unilateral hearing loss. Brain Res. 1342, 24–32.

Dong, S., Mulders, W.H.A.M., Rodger, J., Woo, S., Robertson, D., 2010b. Acoustic trauma evokes hyperactivity and changes in gene expression in guinea-pig auditory brainstem. Eur. J. Neurosci. 31 (9), 1616–1628.
Duan, M., Agerman, K., Ernfors, P., Canlon, B., 2000. Complementary roles of neurotrophin 3 and a N-methyl-D-aspartate antagonist in the protection of noise and aminoglycoside-induced ototoxicity. PNAS 97 (13), 7597–7602.
Duque, D., Pérez-González, D., Ayala, Y.A., Palmer, A.R., Malmierca, M.S., 2012. Topographic distribution, frequency, and intensity dependence of stimulus-specific adaptation in the inferior colliculus of the rat. J. Neurosci. 32 (49), 17762–17774.
Eggermont, J.J., Roberts, L.E., 2004. The neuroscience of tinnitus. Trends Neurosci. 27 (11), 676–682.
Eggermont, J.J., 2013. Hearing loss, hyperacusis, or tinnitus: what is modeled in animal research? Hear Res. 295, 140–149.
Ehret, G., Romand, R. (Eds.), 1997. The Central Auditory System. Oxford University Press, New York, NY.
Furman, A.C., Kujawa, S.G., Liberman, M.C., 2013. Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. J. Neurophysiol. 110 (3), 577–586.
Gu, J.W., Halpin, C.F., Nam, E.-C., Levine, R.A., Melcher, J.R., 2010. Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity. J. Neurophysiol. 104, 3361–3370.
Hickox, A.E., Liberman, M.C., 2014. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? J. Neurophysiol. 111, 552–564.
Knipper, M., Van Dijk, P., Nunes, I., Rüttiger, L., Zimmermann, U., 2013. Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. Prog. Neurobiol. 111, 17–33.
Kujawa, S.G., Liberman, M.C., 2009. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J. Neurosci. 29 (45), 14077–14085.
Langers, D.R.M., De Kleine, E., Van Dijk, P., 2012. Tinnitus does not require macroscopic tonotopic map reorganization. Front Syst. Neurosci. 6 (2).
Liberman, M.C., 1989. Rapid assessment of sound-evoked olivocochlear feedback: suppression of compound action potentials by contralateral sound. Hear Res. 38, 47–56.
Lin, H.W., Furman, A.C., Kujawa, S.G., Liberman, M.C., 2011. Primary neural degeneration in the guinea pig cochlea after reversible noise-induced threshold shift. J. Assoc. Res. Otolaryngol. 12 (5), 605–616.
Ma, W.-L.D., Hidaka, H., May, B.J., 2006. Spontaneous activity in the inferior colliculus of CBA/J mice after manipulations that induce tinnitus. Hear Res. 212 (1–2), 9–21.
Maison, S.F., Liberman, M.C., 2000. Predicting vulnerability to acoustic injury with a noninvasive assay of olivocochlear reflex strength. J. Neurosci. 20 (12), 4701–4707.
Milbrandt, J.C., Holder, T.M., Wilson, M.C., Salvi, R.J., Caspary, D.M., 2000. GAD levels and muscimol binding in rat inferior colliculus following acoustic trauma. Hear Res. 147 (1–2), 251–260.
Mulders, W.H.A.M., Robertson, D., 2011. Progressive centralization of midbrain hyperactivity after acoustic trauma. Neuroscience 192, 753–760.
Mulders, W.H.A.M., Robertson, D., 2009. Hyperactivity in the auditory midbrain after acoustic trauma: dependence on cochlear activity. Neuroscience 164 (2), 733–746.
Niu, Y., Kumaraguru, A., Wang, R., Sun, W., 2013. Hyperexcitability of inferior colliculus neurons caused by acute noise exposure. J. Neurosci. Res. 91 (2), 292–299.
Salvi, R.J., Hamernik, R.P., Henderson, D., 1978. Discharge patterns in the cochlear nucleus of the chinchilla following noise induced asymptotic threshold shift. Exp. Brain Res. 32 (3), 301–320.
Salvi, R.J., Saunders, S.S., Gratton, M.A., Arehole, S., Powers, N., 1990. Enhanced evoked response amplitudes in the inferior colliculus of the chinchilla following acoustic trauma. Hear Res. 50 (1–2), 245–258.
Salvi, R.J., Wang, J., Ding, D., 2000. Auditory plasticity and hyperactivity following cochlear damage. Hear Res. 147 (1–2), 261–274.
Schaette, R., McAlpine, D., 2011. Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. J. Neurosci. 31 (38), 13452–13457.
Scholl, B., Wehr, M., 2008. Disruption of balanced cortical excitation and inhibition by acoustic trauma. J. Neurophysiol. 100, 646–656.
Sun, W., Deng, A., Jayaram, A., Gibson, B., 2012. Noise exposure enhances auditory cortex responses related to hyperacusis behavior. Brain Res. 1485, 108–116.
Szczepaniak, W.S., Møller, A.R., 1995. Evidence of decreased GABAergic influence on temporal integration in the inferior colliculus following acute noise exposure: a study of evoked potentials in the rat. Neurosci. Lett. 196, 77–80.
Wang, J., Salvi, R.J., Powers, N., 1996. Plasticity of response properties of inferior colliculus neurons following acute cochlear damage. J. Neurophysiol. 75 (1), 171–183.
Willott, J.F., Lu, S.M., 1982. Noise-induced hearing loss can alter neural coding and increase excitability in the central nervous system. Science 216 (4552), 1331–1332.
Zeng, F.-G., 2013. An active loudness model suggesting tinnitus as increased central noise and hyperacusis as increased nonlinear gain. Hear Res. 295, 172–179.
Zheng, X.-Y., Henderson, D., Hu, B.-H., Ding, D.-L., McFadden, S.L., 1997. The influence of the cochlear efferent system on chronic acoustic trauma. Hear Res. 107, 147–159.
Zuccotti, A., Kuhn, S., Johnson, S.L., Franz, C., Singer, W., Hecker, D., Knipper, M., 2012. Lack of brain-derived neurotrophic factor hampers inner hair cell synapse physiology, but protects against noise-induced hearing loss. J. Neurosci. 32 (25), 8545–8553.